



Recombinant com1 Attachment to Polystyrene Beads: A Surface Display Mimic for *C. burnetii*

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Biological Agent Simulants

- **Need for biological simulants (point detection)**
 - Actual biological materials release is dangerous and restricted by law
- **Replace live agent but maintain agent properties:**
 - Physical (size, aerosolization...)
 - Detection (surface antigens)

**Joint Biological Point
Detection System
(JBPDS)**





Current Simulants Used for Field Tests

- *Bacillus atrophaeus* (BG)--Gram-pos. spore former
- *Erwinia herbicola* (EH)--Gram-neg. vegetative
- Male-specific bacteriophage type 2 (MS2)--viral
- Ovalbumin (OV)--protein toxin

Good preservation of many physical characteristics

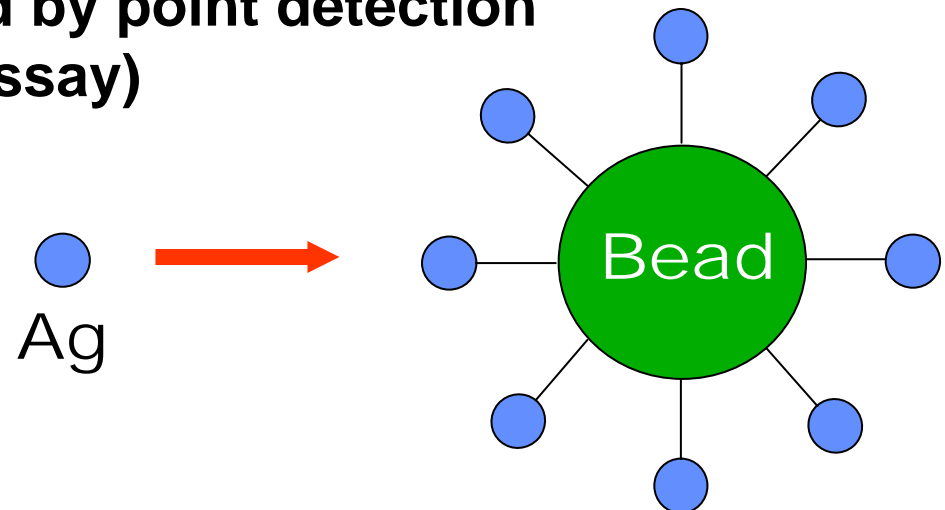
Detection properties (surface antigens) not represented

Need more realistic simulants for point detection testing



Bead Technology Approach

- Identify agent-specific surface antigen
- Produce antigen (recombinant)
- Attach recombinant antigen to surface of a microsphere (polystyrene bead)
 - Bead simulant mimics surface of agent
 - Potentially detected by point detection systems (immunoassay)





Bead Simulant for *Coxiella burnetii*

- **Coxiella Outer Membrane Protein 1 (com1)**
 - **Surface protein of *Coxiella burnetii***
 - **Most immunodominant protein**
 - **Unique to *Coxiella***
 - **Good target antigen candidate**



Bead Simulant Project Goals

Test feasibility of attaching recombinant antigen to polystyrene beads

- **Test two different approaches for antigen attachment**
 - **Passive adsorption (hydrophobic)**
 - **Covalent linkage (covalent bond)**
- **Beads bind com1 antigen?**
- **Bound com1 recognized by α -*Coxiella* Abs?**
(Bead simulant mimic *Coxiella* surface?)



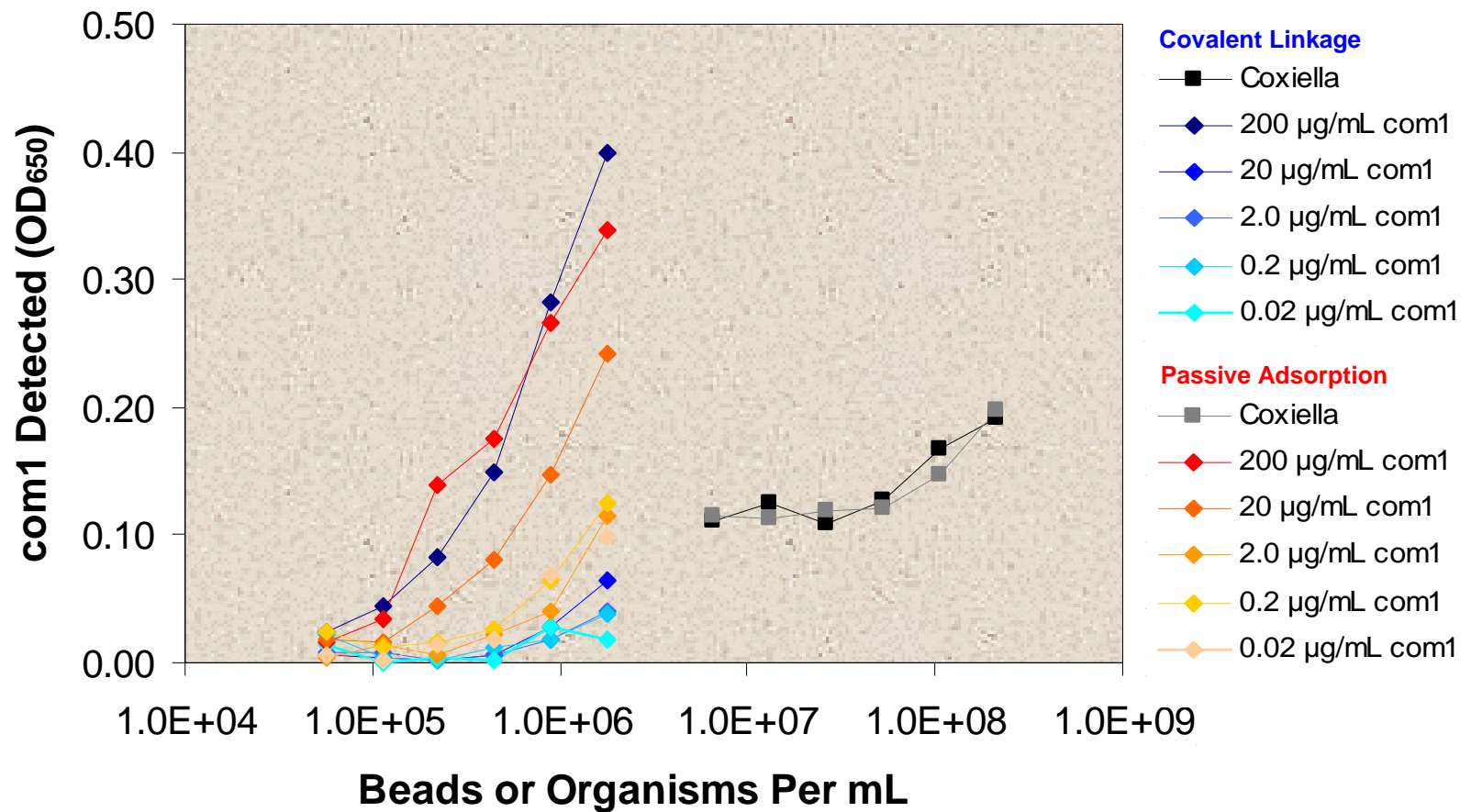
ELISA

1. Determine if com1 attached to beads
 1. Passive adsorption
 2. Covalent coupling
2. Compare antigenicity of com1 via passive or covalent attachment to beads vs. *Coxiella*
 - 1° Antibody: α -com1 mono/polyclonal antibody
 - 2° Antibody: conjugated to HRP



Detection of com1 Attached to Beads by Passive Adsorption and Covalent Linkage

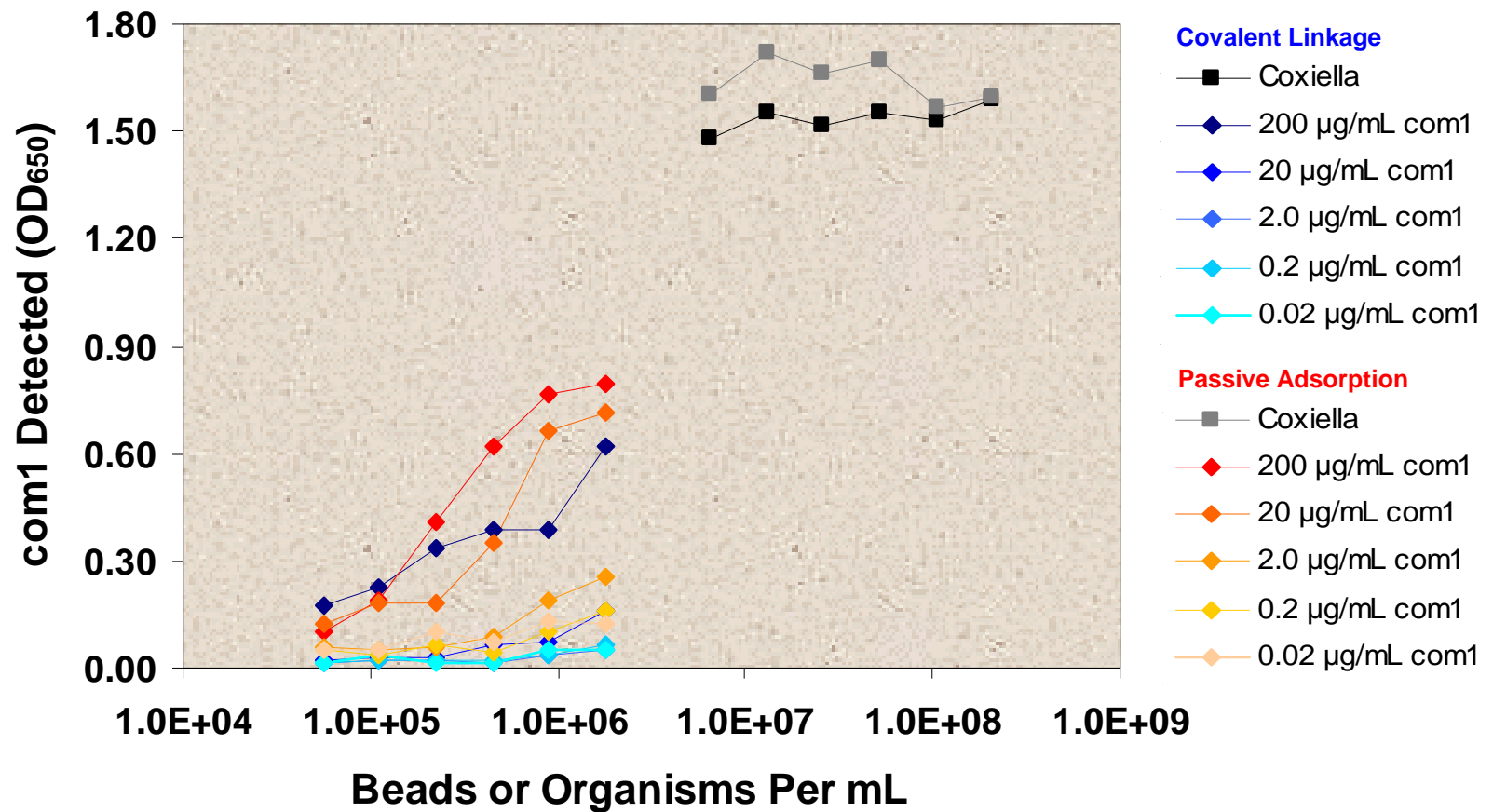
α -com1 Monoclonal Antibody





Detection of com1 Attached to Beads by Passive Adsorption and Covalent Linkage

α -*Coxiella* Polyclonal Antibody (PEO-CBD)





Flow Cytometry

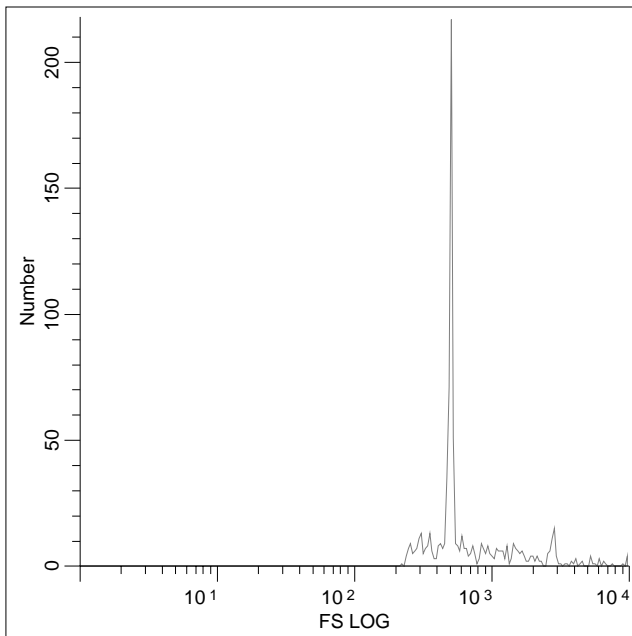
1. Compare size distribution of passive and covalent beads vs. *Coxiella*
 - “Forward scatter” (relative size)
2. Compare com1 binding distribution across each bead population vs. *Coxiella*
 - Relative fluorescence
 - 1° Antibody: α -com1 monoclonal antibody
 - 2° Antibody: conjugated to FITC



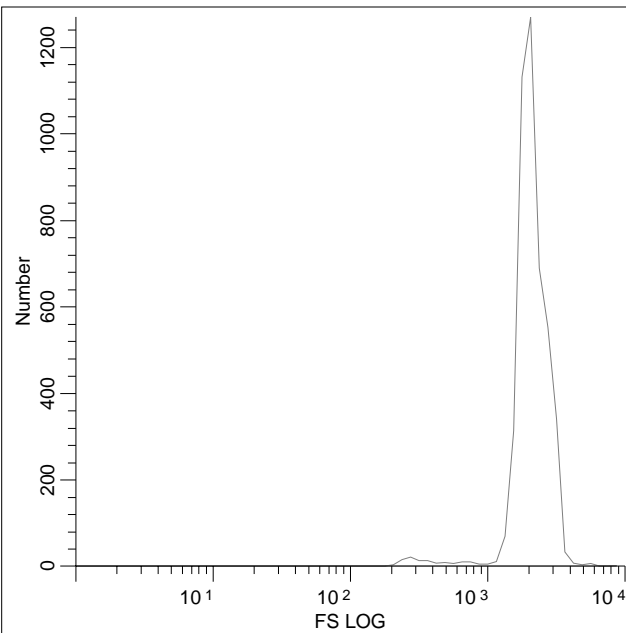
Flow Cytometry of Polystyrene Beads

Relative Size Distribution (FS)

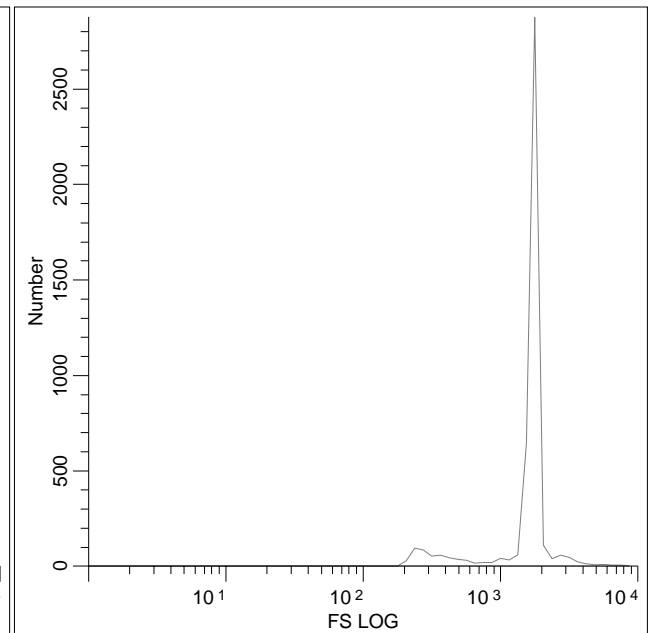
Coxiella



Passive Adsorption Beads



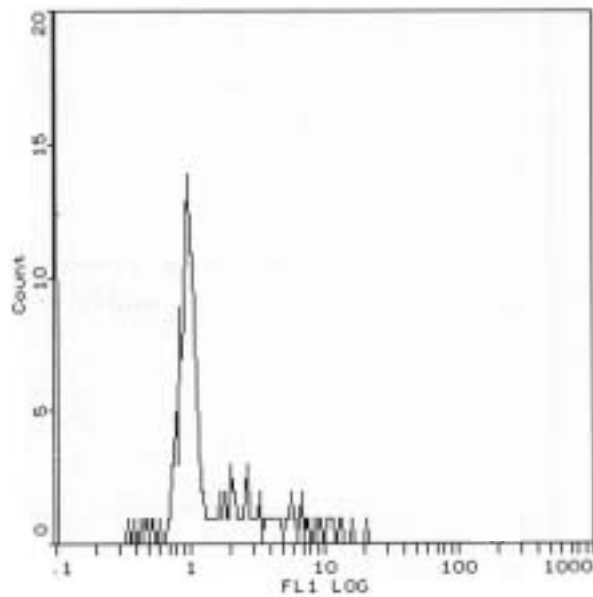
Covalent Coupling Beads



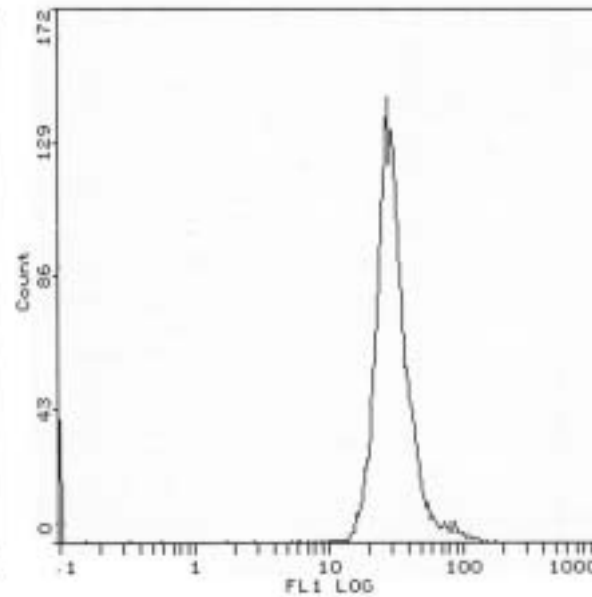


Flow Cytometry of Polystyrene Beads Relative Fluorescence (FL)

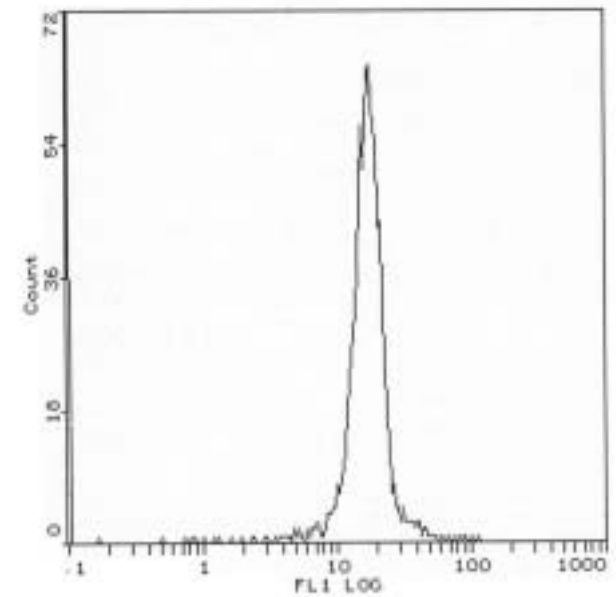
Coxiella



Passive Adsorption Beads



Covalent Coupling Beads



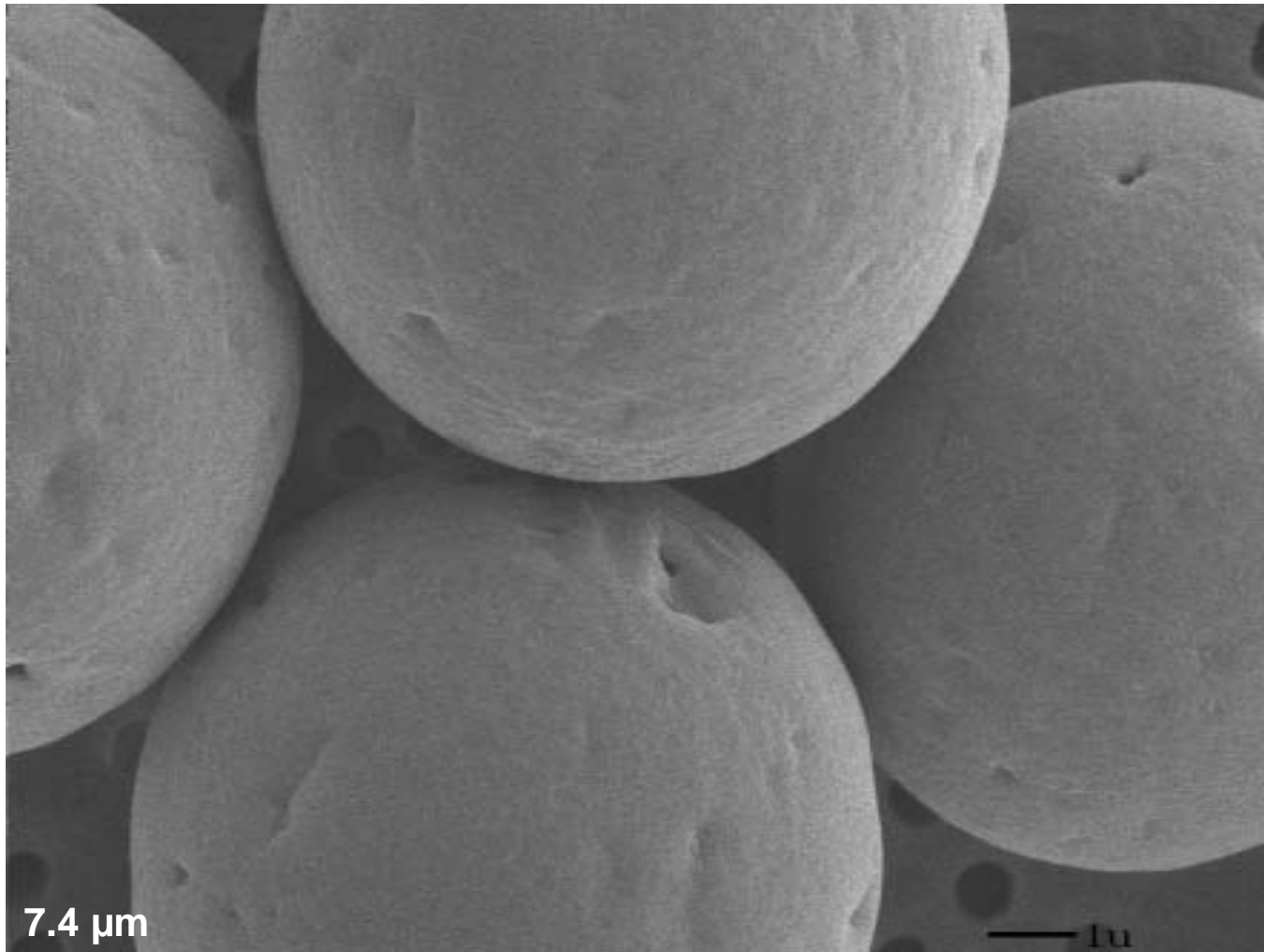


Immuno Scanning Electron Microscopy

- **Compare uniformity of com1 attachment on individual beads (passive vs. covalent)**
 - **1° Antibody: α -com1 monoclonal antibody**
 - **2° Antibody: conjugated to colloidal gold (25 nm)**

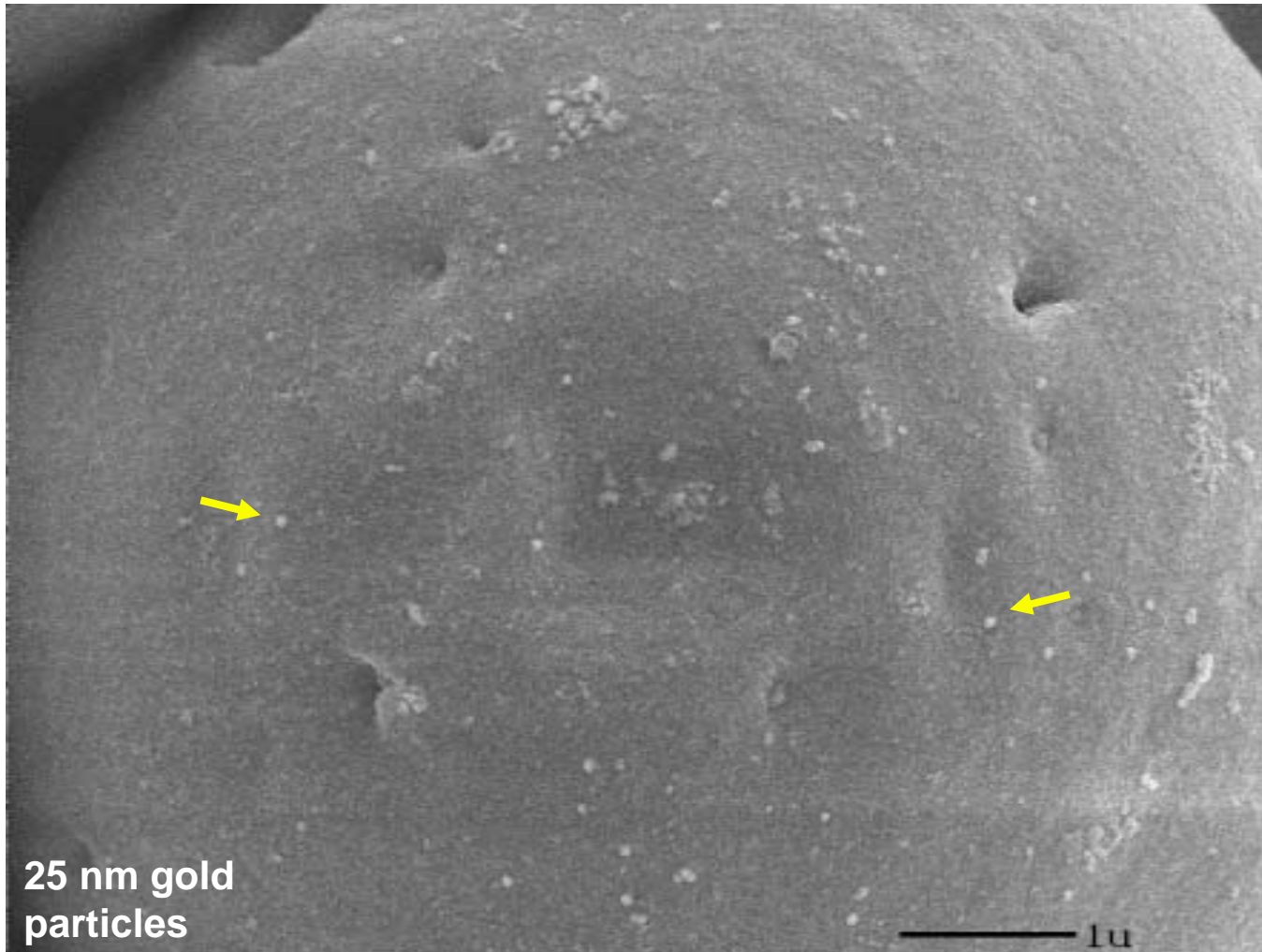


Covalent Coupling Beads—No com1



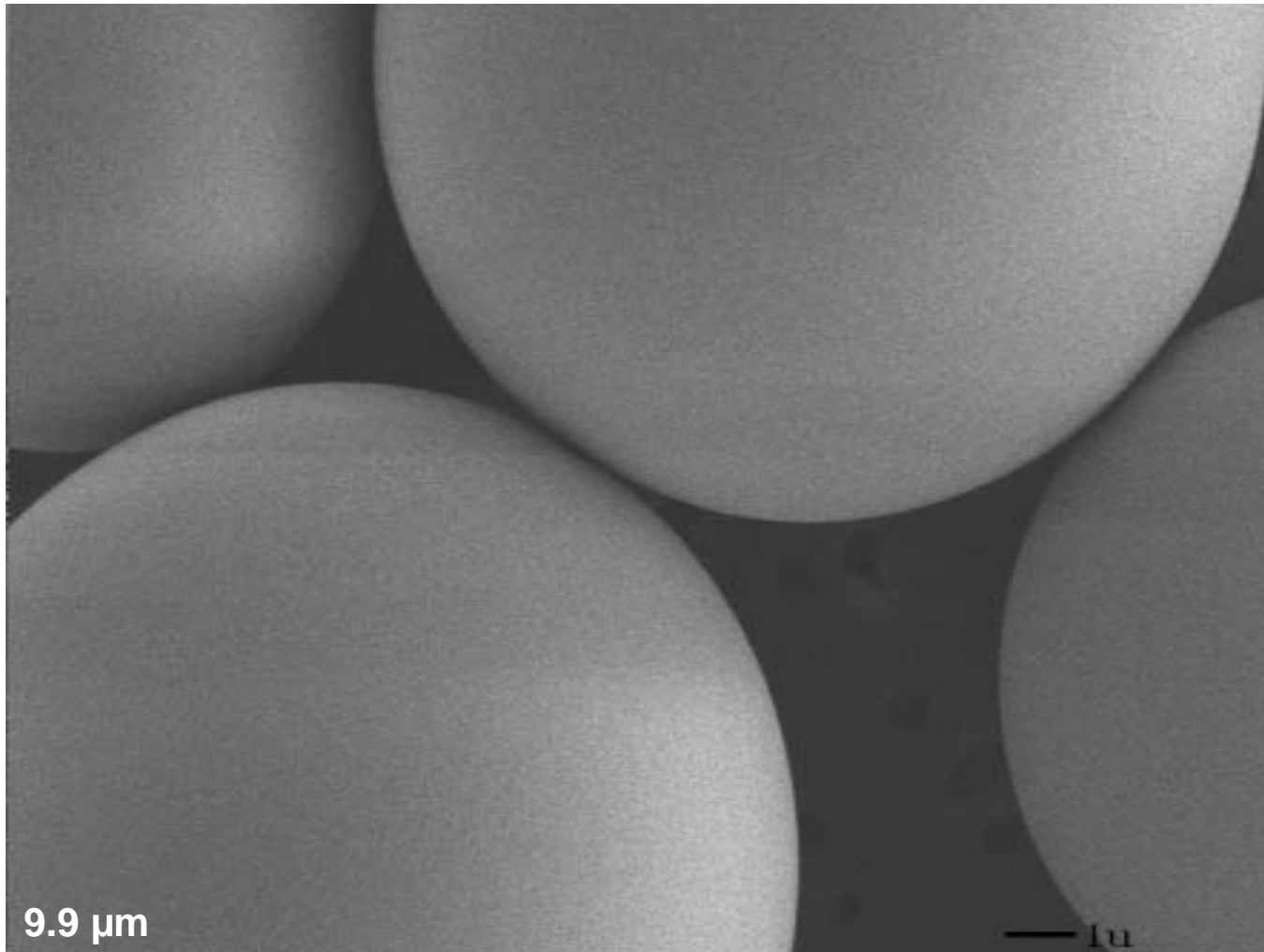


Covalent Coupling Beads—w/ com1



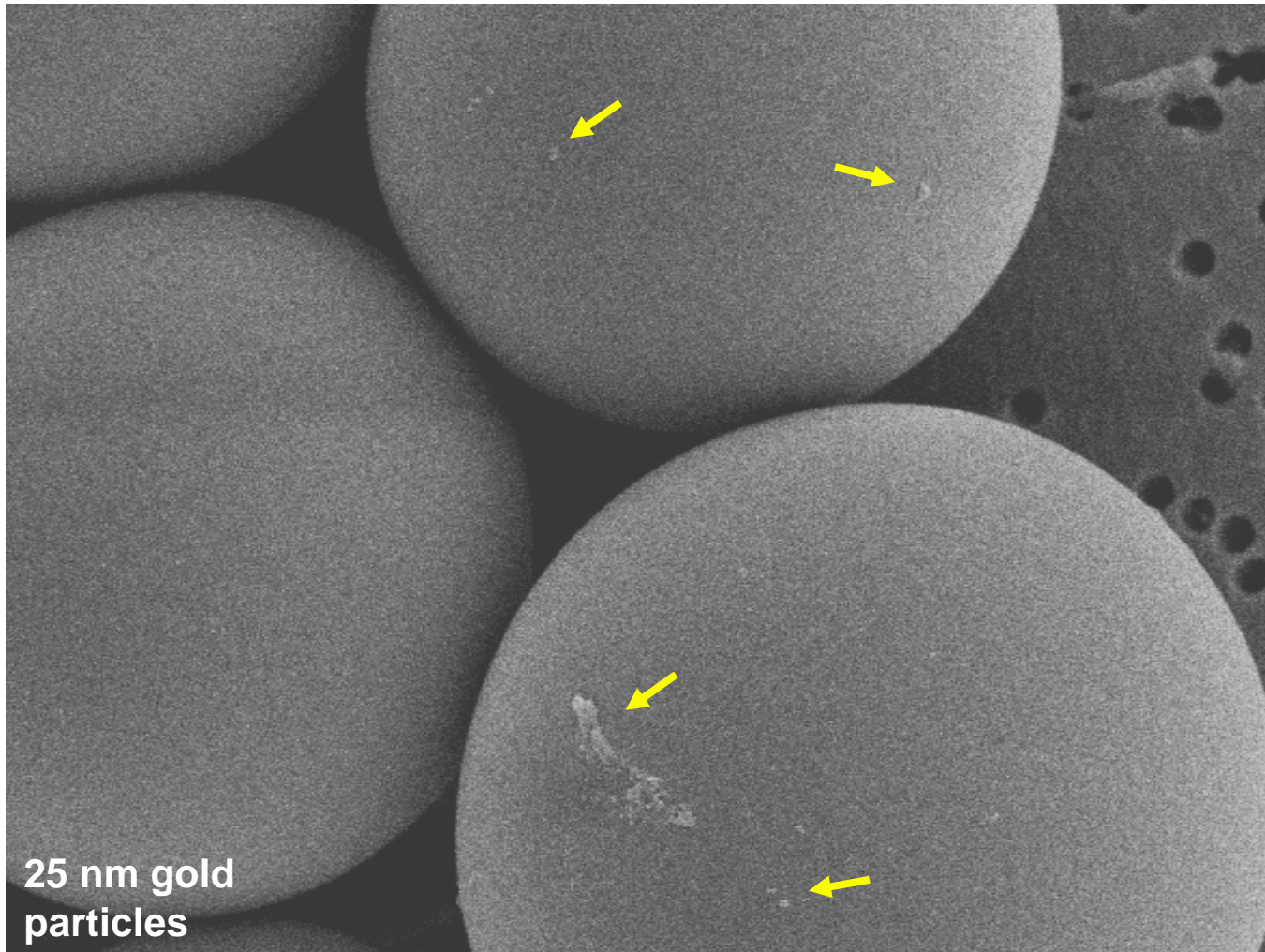


Passive Adsorption Beads—No com1



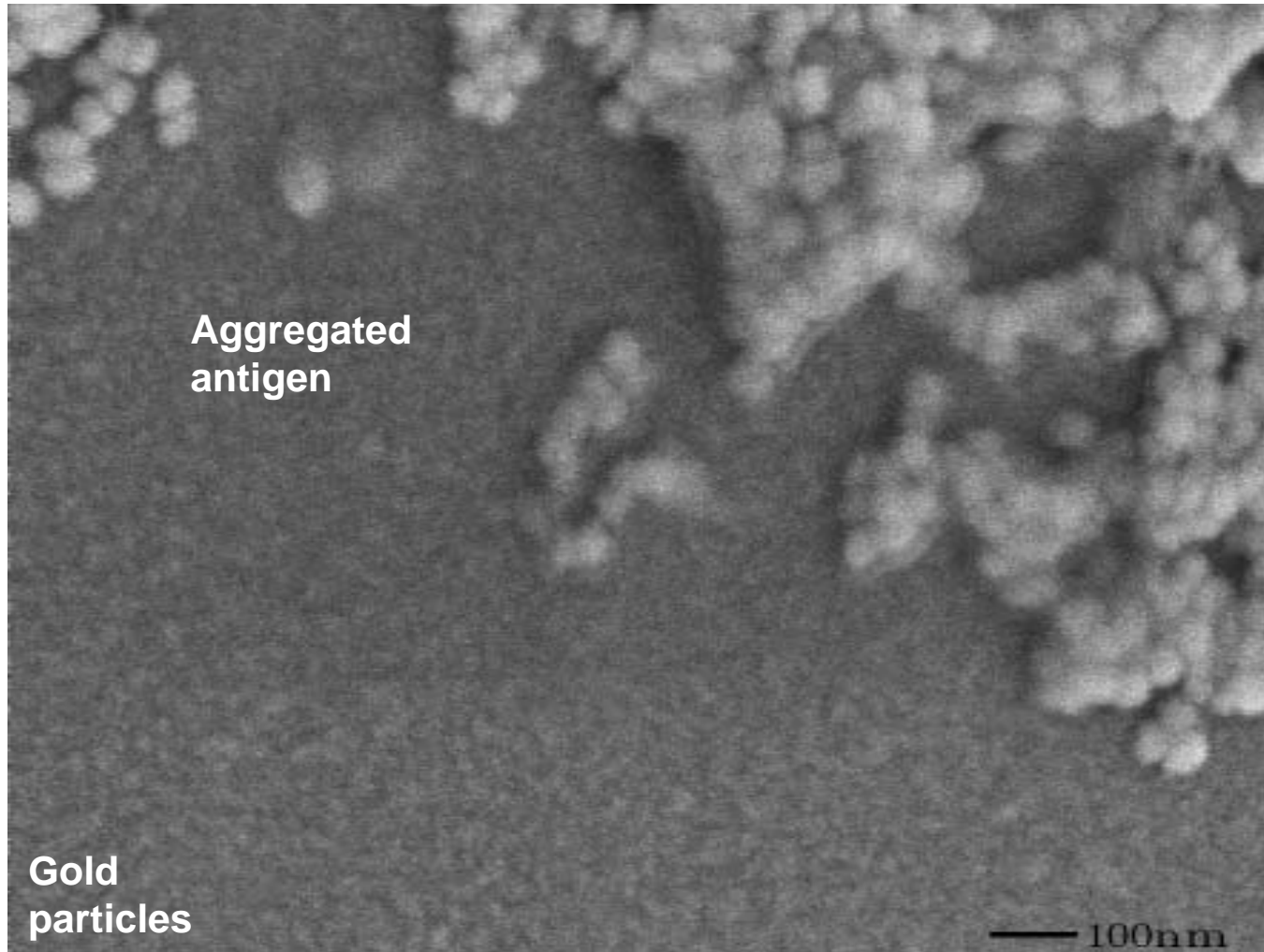


Passive Adsorption Beads—w/ com1





Passive Adsorption Beads—w/ com1





SUMMARY

- **Bead technology is a rapid, functional approach for producing biosimulants for any biological agent**
 - **Surface antigenicity of agent is mimicked**
 - **Physical characteristics are customizable**
- **Produced two bead simulants for *C. burnetii***
 - **Recognized by both poly/monoclonal antibodies**
 - **Levels of bead (com1) detection similar to *Coxiella***
 - **Distribution of com1 across bead populations similar to *Coxiella***
 - **Good surface antigen mimic of *Coxiella***



ACKNOWLEDGEMENTS

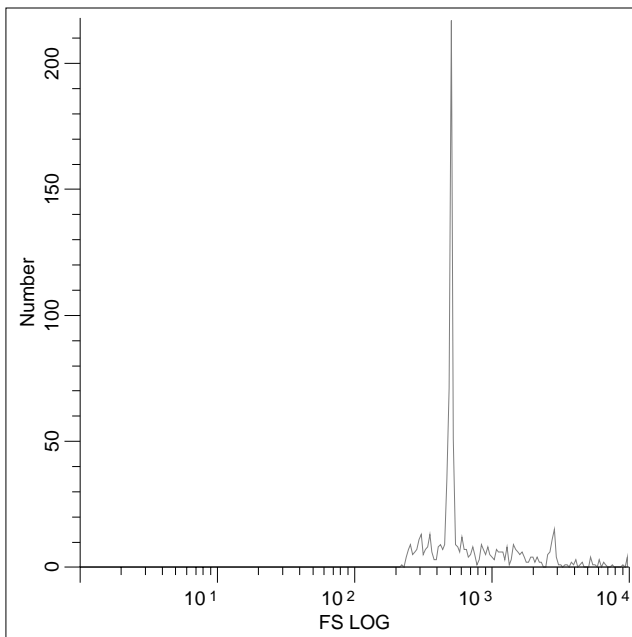
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Flow Cytometry of Polystyrene Beads

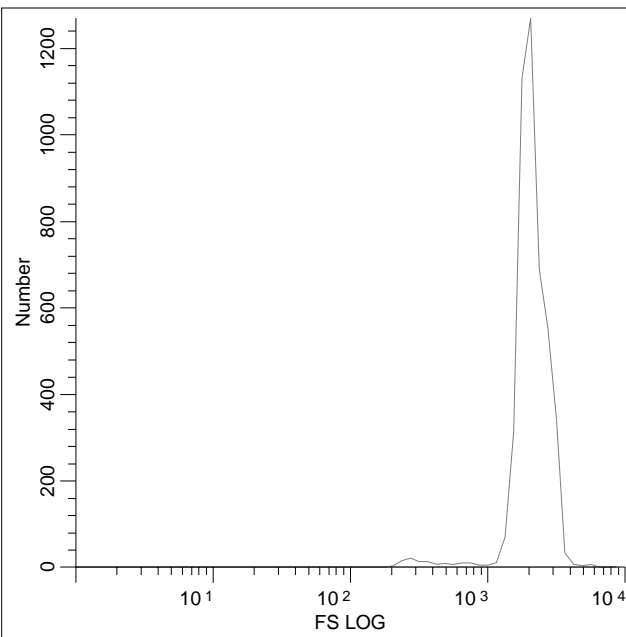
Relative Size Distribution (FS)

Coxiella



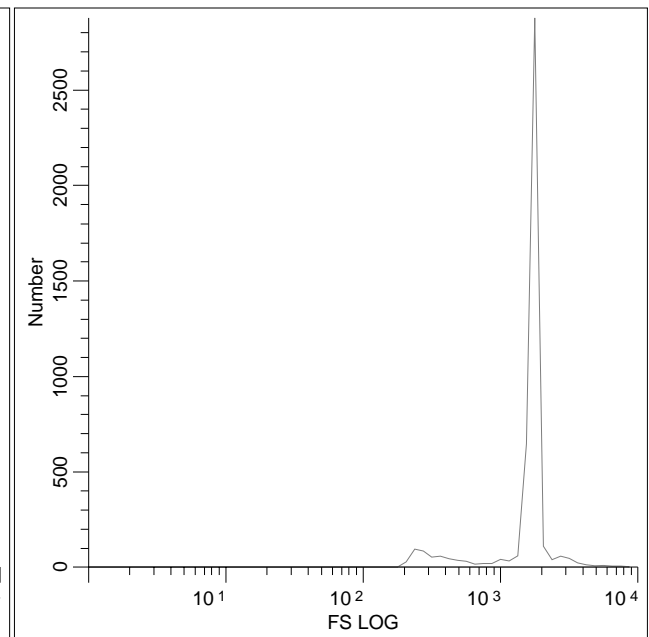
Actual Size: 0.5 μm

Passive Adsorption Beads



9.9 μm

Covalent Coupling Beads



7.4 μm



Biological Point Detection Systems

- Real-time detection of biological agents (on-site)
- Components:
 - Detector/Trigger (BAWS)
 - Collector (Cyclone)
 - Fluid transfer system
 - Identifier (PCR, immunoassay)
- Field-tested with simulants



Joint Biological Point Detection System
(JBPDS)



Two approaches for antigen attachment:

- **Passive adsorption**
 - Attachment due to hydrophobic interactions
 - Positives: fast, easy, relatively inexpensive
 - Negatives: temporary, less stable, antigen partially unfolded (still antigenic?)
- **Covalent coupling**
 - Antigen attached via covalent bonding
 - Positives: permanent, more stable, native (antigenic)
 - Negatives: complex, more expensive procedure

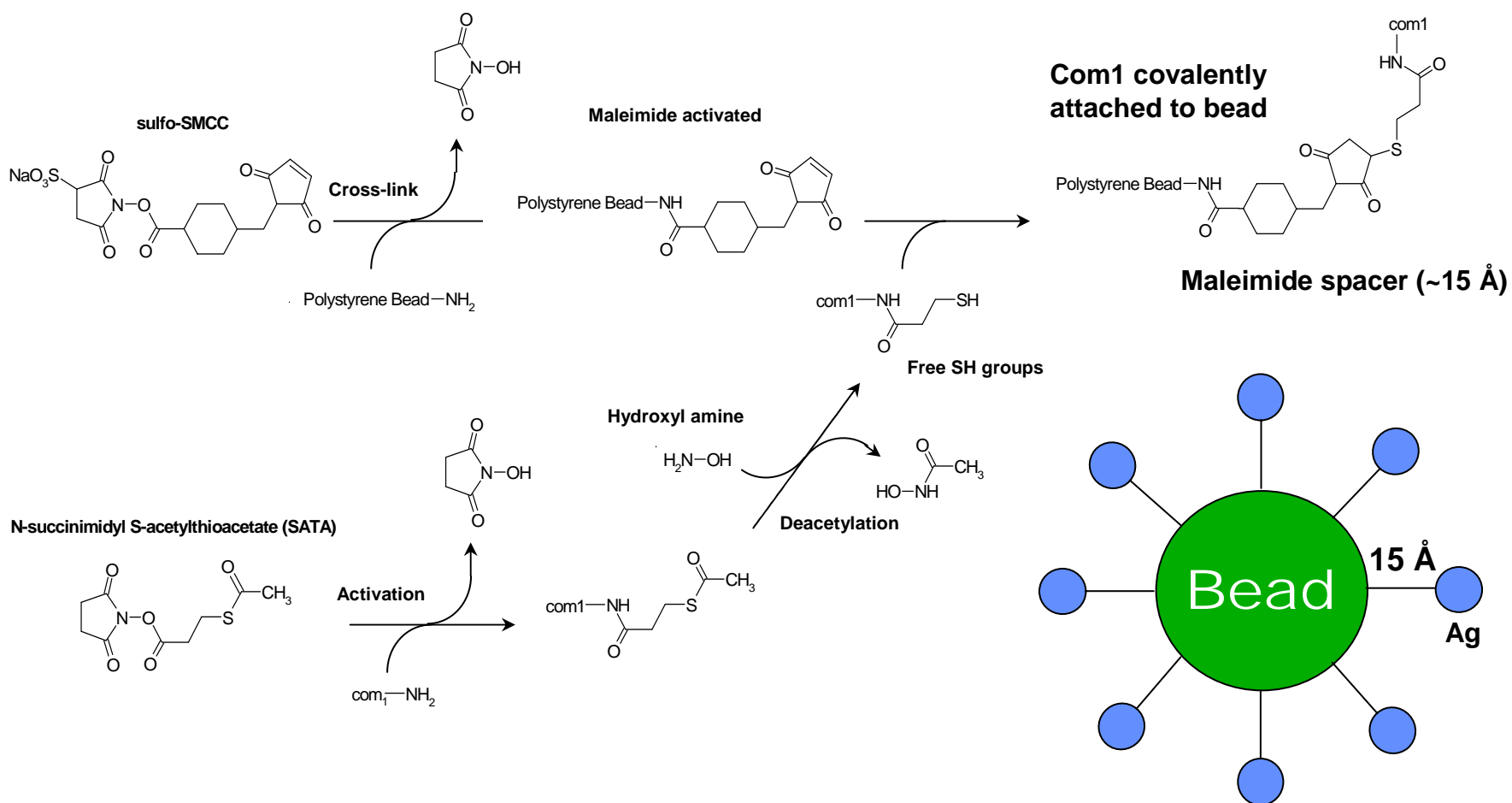


Benefits of Bead Technology

- Fast, easy—only requires agent-specific recombinant antigen
- Customize size and surface antigens
 - Mimics physical AND detection properties
- Can be potentially used to simulate any biological agent:
 - Bacteria—*B. anthracis*...
 - Viruses—VEE...
 - Toxins—Ricin...



Covalent Attachment of com1 to NH₂-Modified Polystyrene Beads

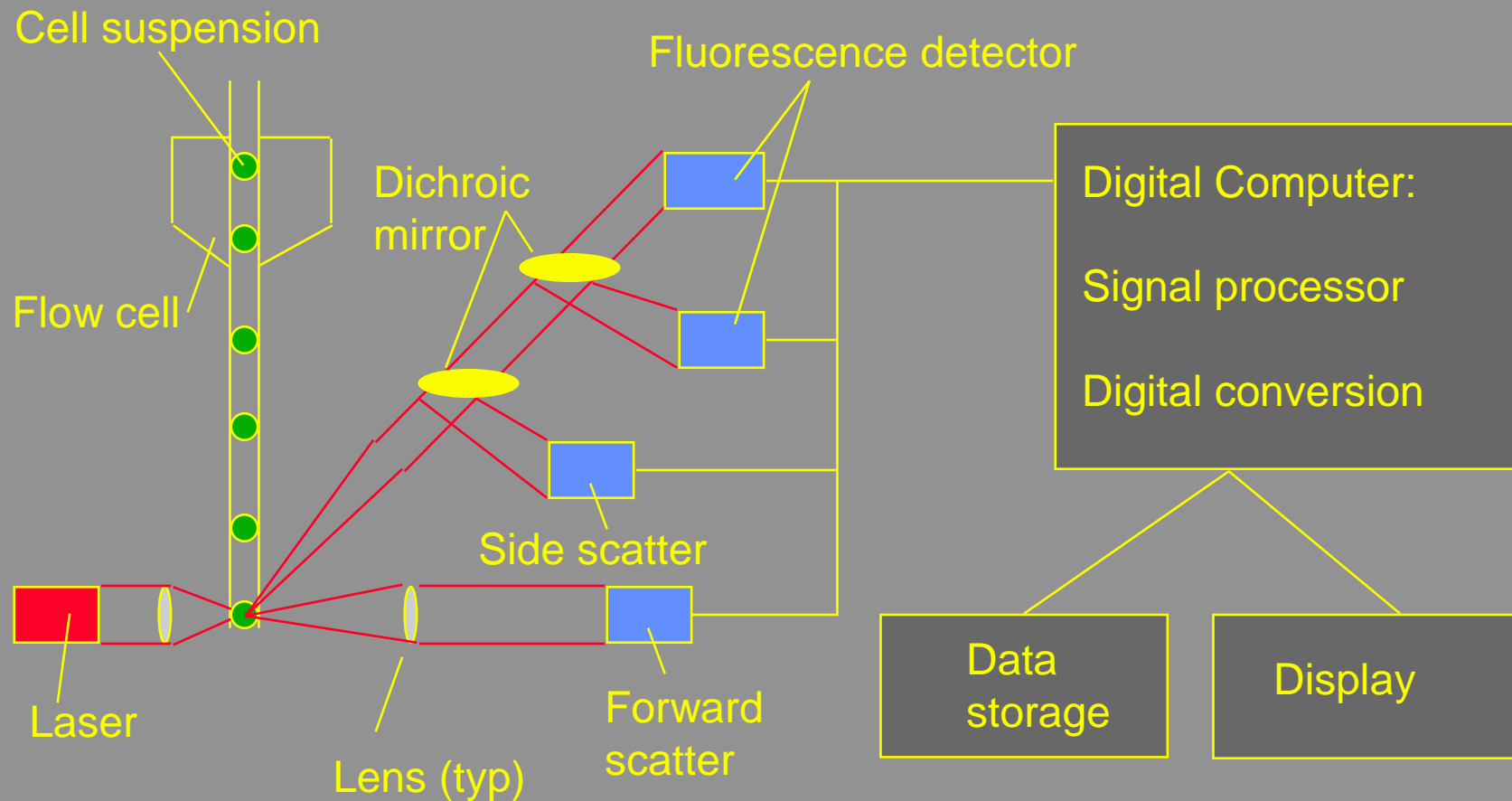




Summary--ELISA

- **com1 attached to beads (adsorbed and covalent)**
- **Mono/polyclonal antibodies detect com1 on both bead preps similar to *Coxiella***
- **Both bead preps sufficient *Coxiella* mimics (surface antigenicity)**

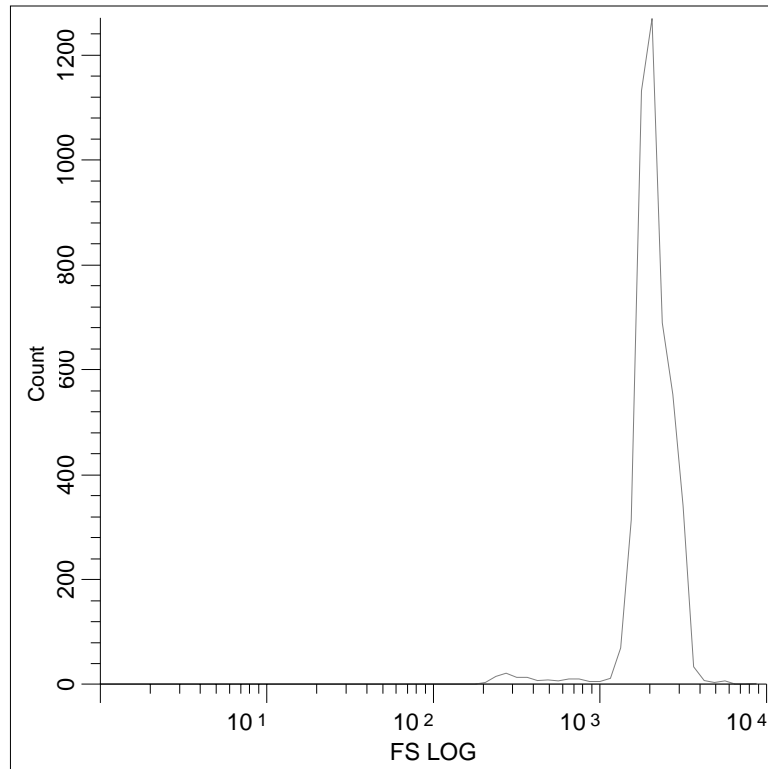
Basic Components of Flow Cytometry



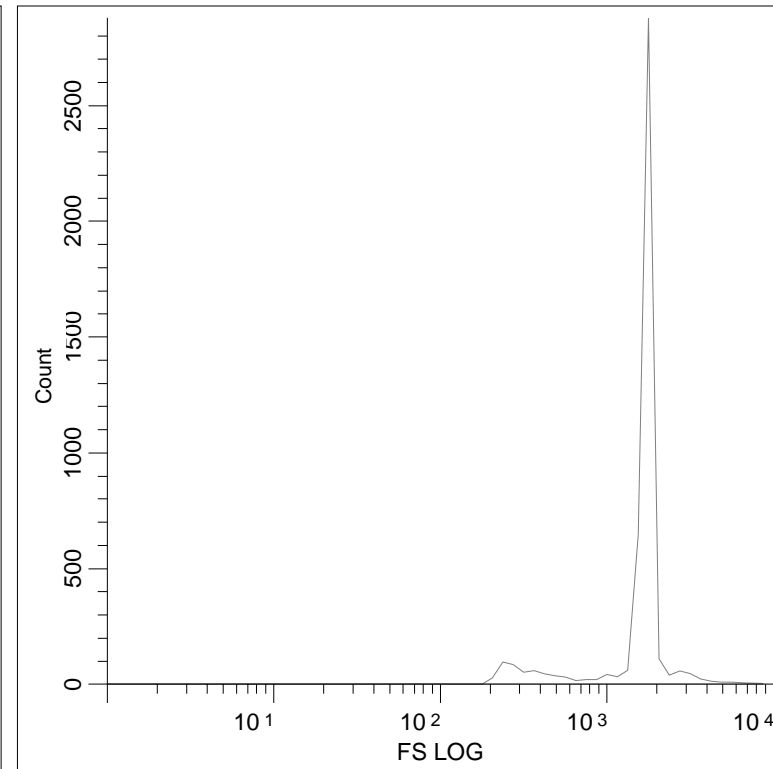


Flow Cytometry of Polystyrene Beads Relative Size Distribution (FS)

Passive Adsorption Beads



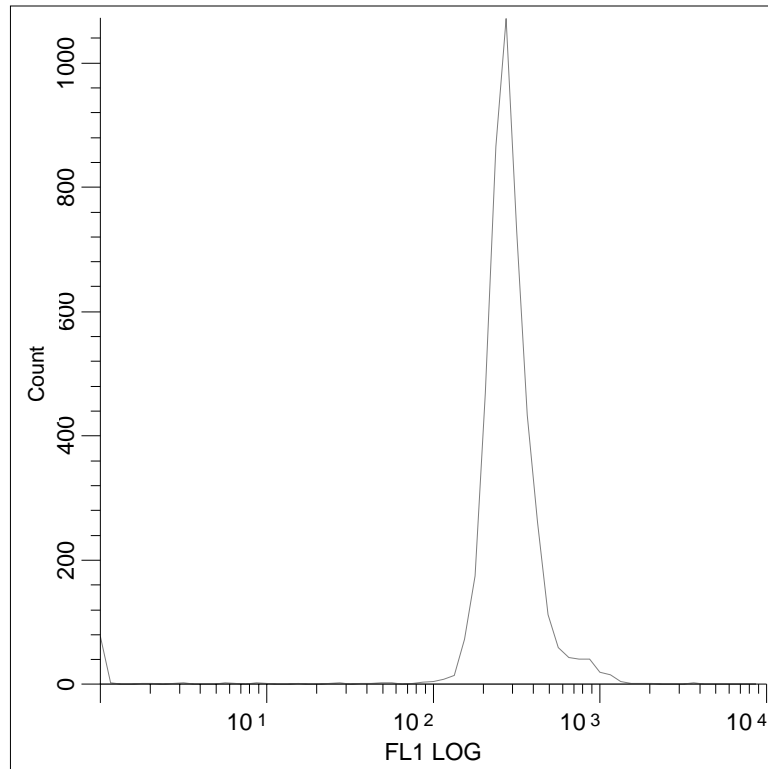
Covalent Coupling Beads



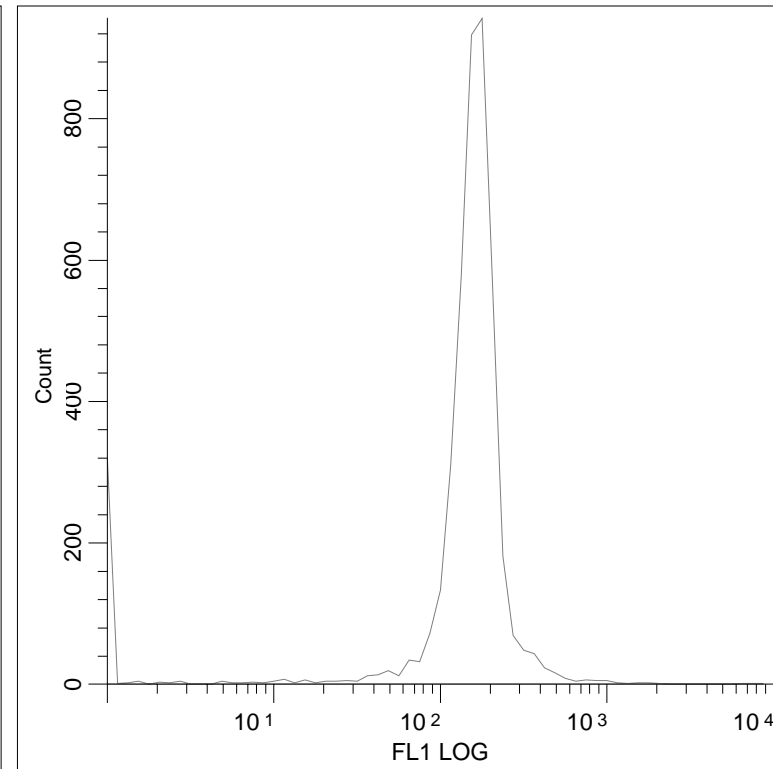


Flow Cytometry of Polystyrene Beads Relative Fluorescence (FL) Distribution

Passive Adsorption Beads



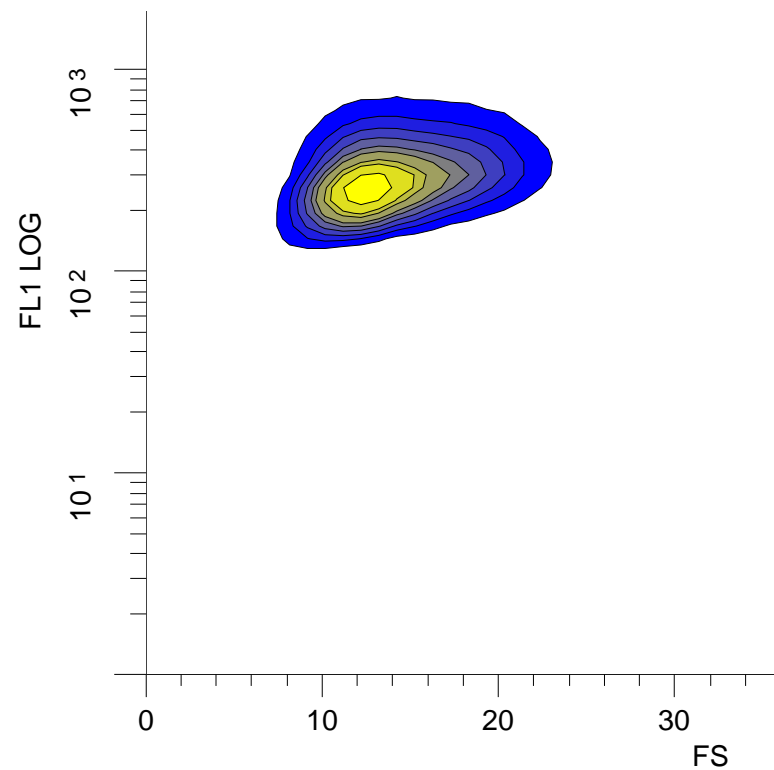
Covalent Coupling Beads



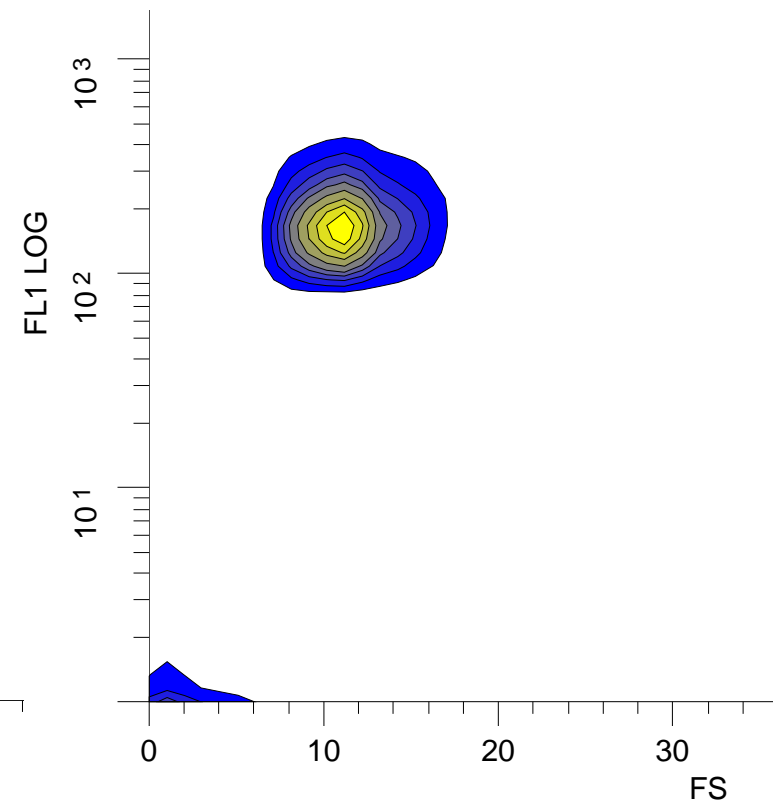


Flow Cytometry of Polystyrene Beads—SS vs FS

Passive Adsorption Beads



Covalent Coupling Beads



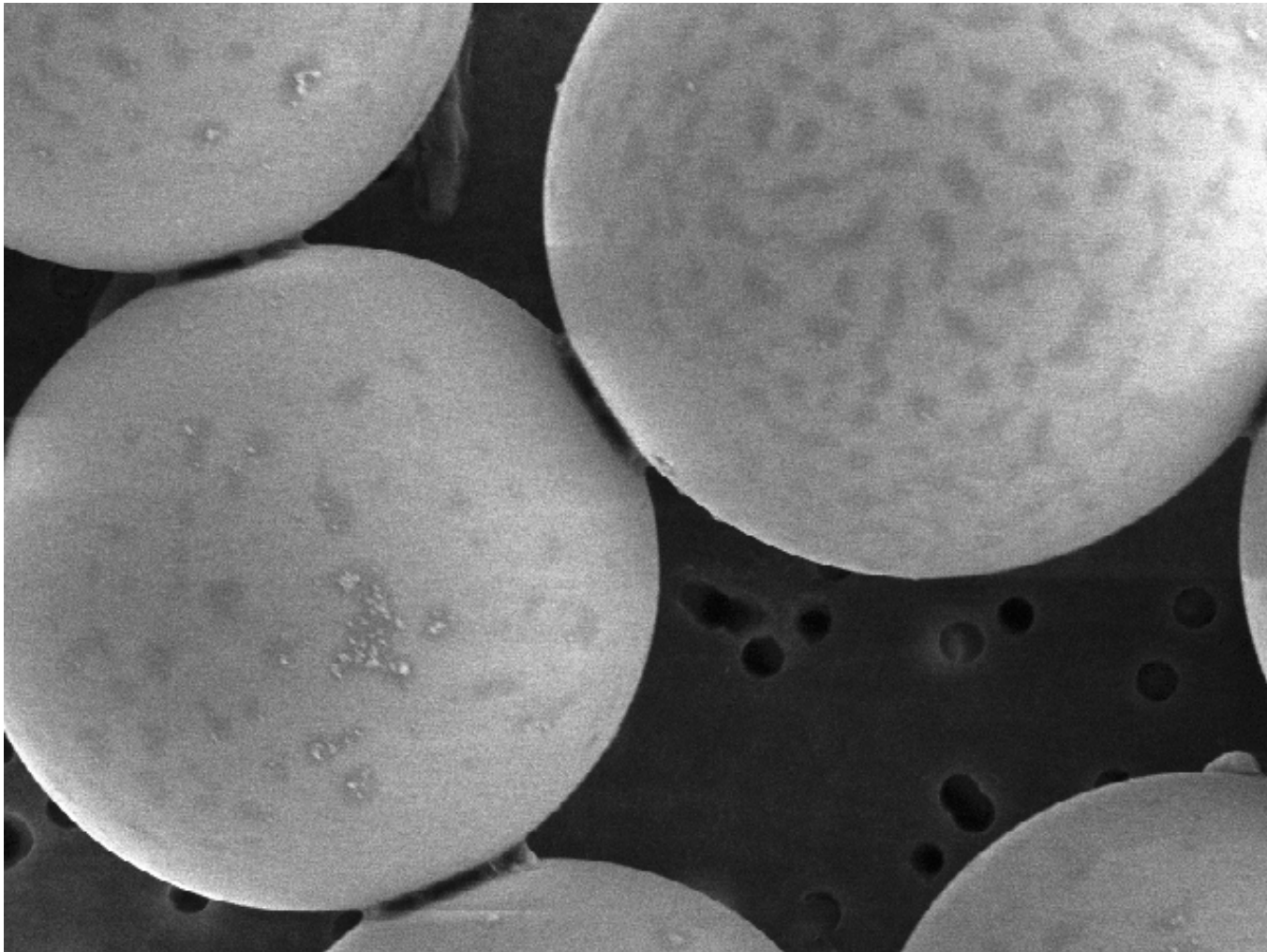


Summary--Flow Cytometry

- Size distribution of passive adsorption beads is wider than *Coxiella*; covalent bead size distribution similar to *Coxiella*
 - Controllable
- com1 binding (fluorescence) distribution is similar among both bead populations and *Coxiella*

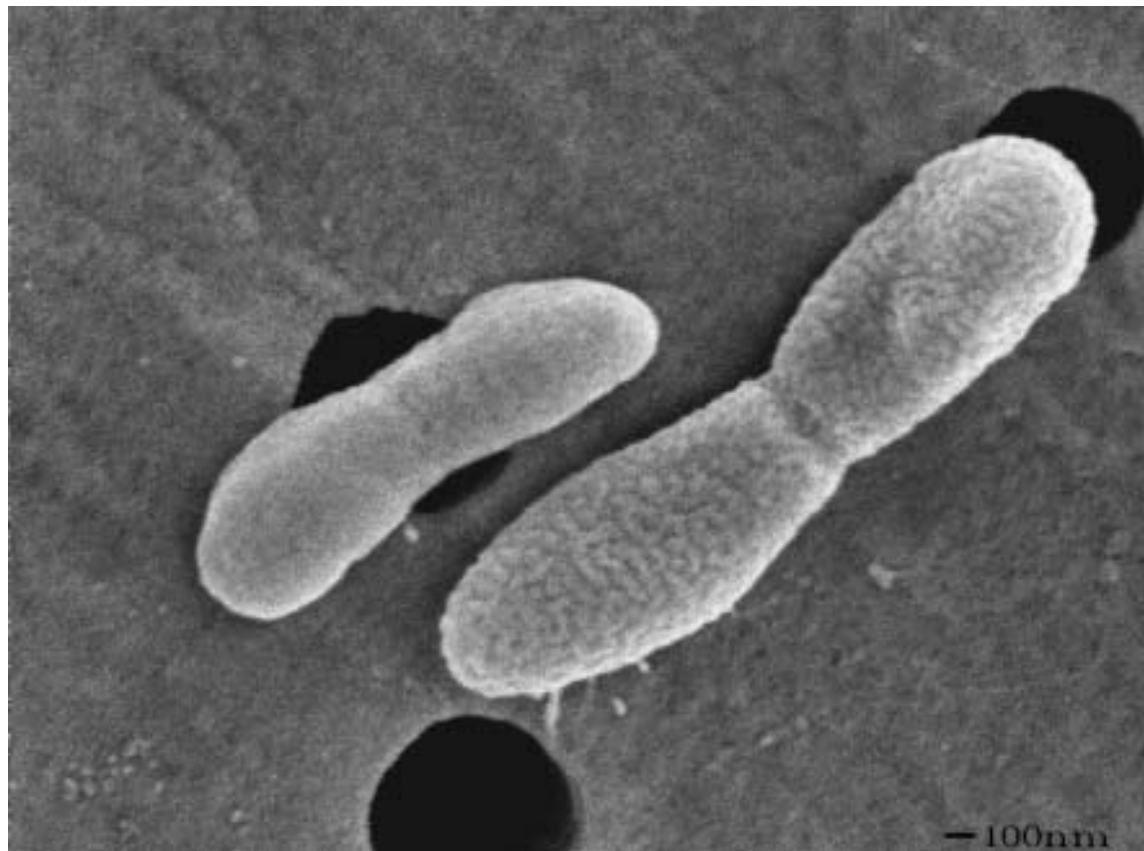


Passive Adsorption Beads—w/ com1



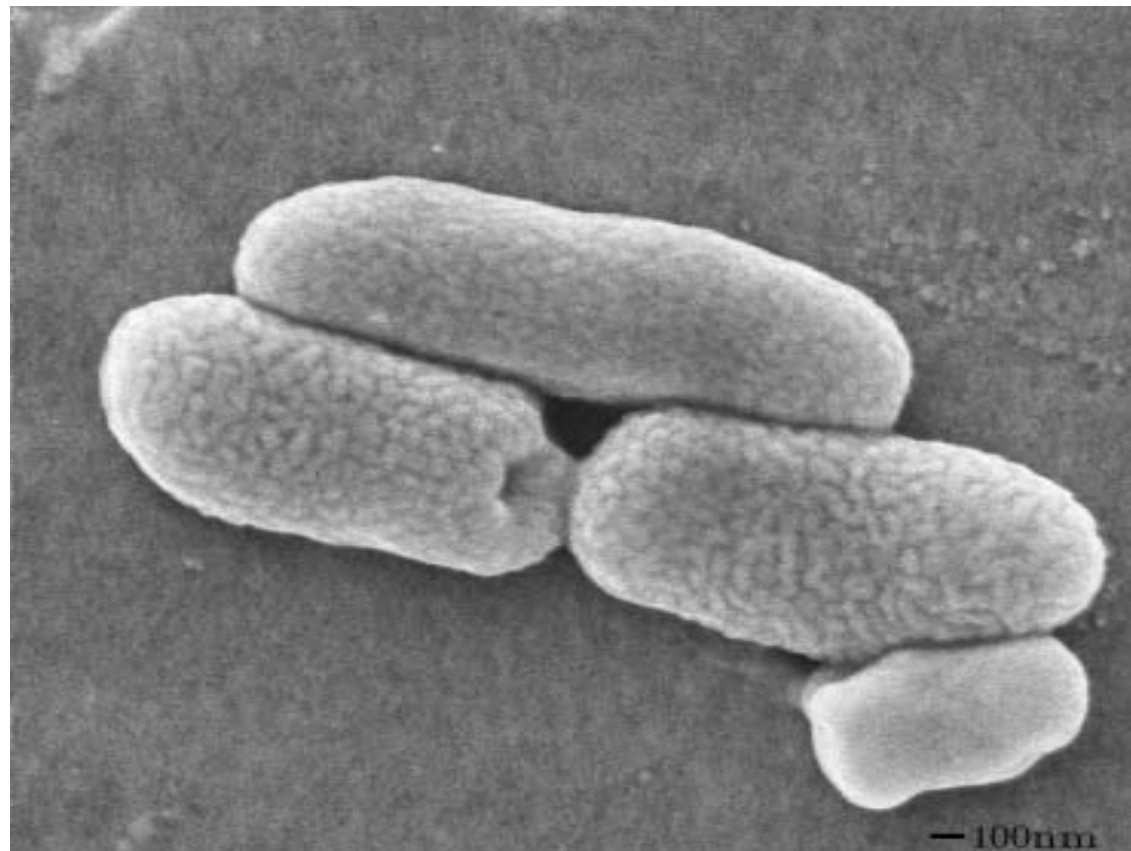


***Coxiella burnetii*—No Antibody**





***Coxiella burnetii*— α com1 Monoclonal Antibody**





Summary--Immuno SEM

- **Uniform coating of com1 on covalent beads**
- **com1 “clumped” (aggregated) on passive adsorption beads**
 - **Does not significantly affect com1 antigenicity**



Poly and Monoclonal Antibody Affinity for com1

Antibody	Antigen	K_D (M)
Poly pDPG	Coxiella	1.2E-08
		2.7E-08
		3.7E-09
		1.4E-08
Poly pDPG	com1	3.7E-08
		2.9E-09
		8.1E-10
		1.4E-08
Mono 11B24	Coxiella	1.7E-09
		3.7E-09
		4.0E-10
		3.2E-09
Mono 13A44	com1	2.2E-09
		3.4E-09
		4.0E-09
		3.5E-09
Mono 13A44	com1	3.8E-09
		3.7E-09
		3.7E-09
		3.7E-09



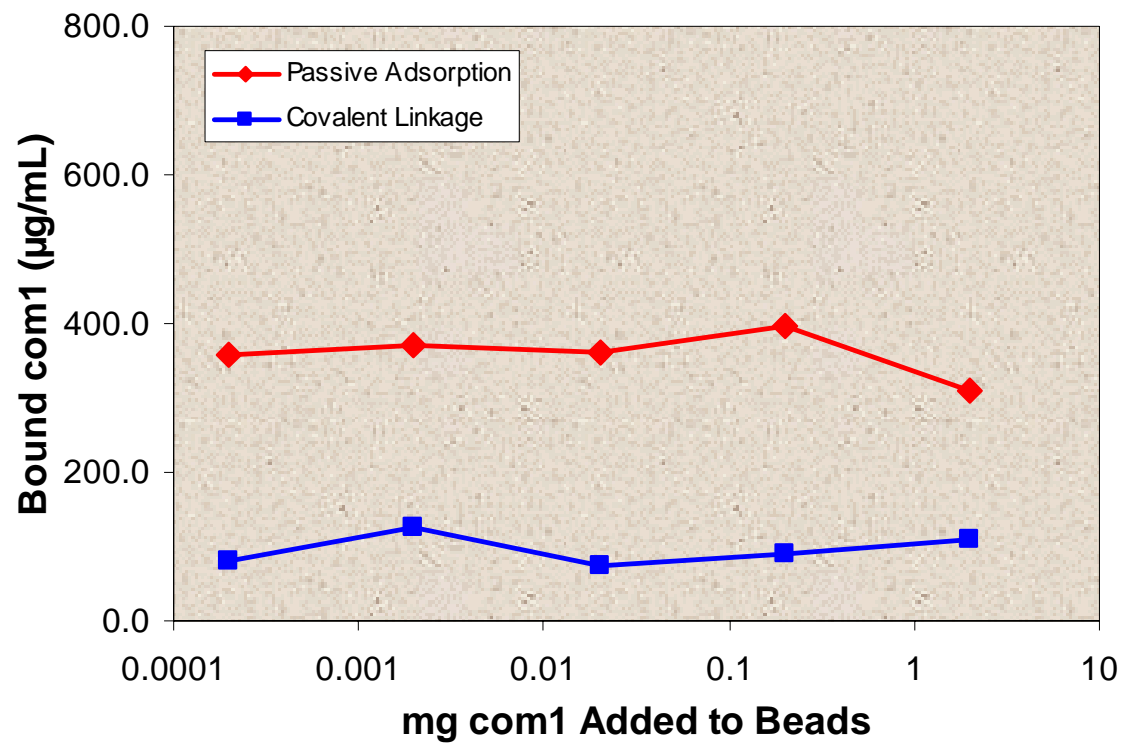
Antibody Avidity/Affinity for *C. burnetii* and com1

Antibody	Class	Antigen	K _D (M)
pDPG	Polyclonal	Coxiella	1.95E-08
	Polyclonal	com1	2.00E-08
11B24/13A44	Monoclonal	Coxiella	2.24E-09
	Monoclonal	com1	3.68E-09

- Purified com1 binds poly/monoclonal Ab similar to killed *C. burnetii* samples
- com1 is a good mimic for *C. burnetii*

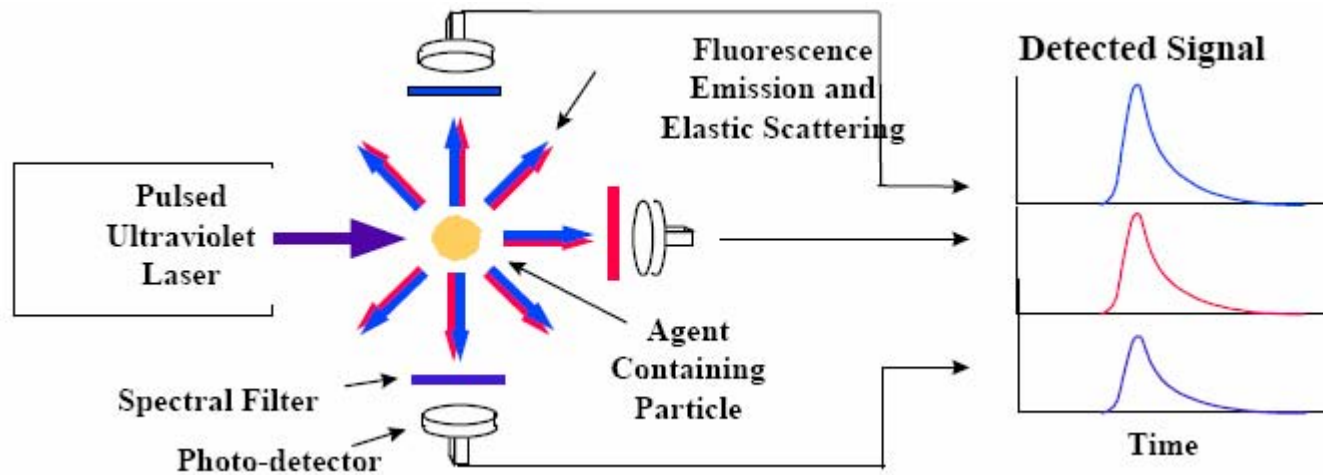


Concentration of com1 on Polystyrene Beads





Three Channel Sensor (BAWS)



Elastic Scattering and Fluorescence Emission Spectrum

